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Diversity in the Genera *Avitellina* and *Thysaniezia* (Cestoda: Cyclophyllidea): Genetic Evidence

C. T. BA,[1] X. Q. WANG,[2] F. RENAUD,[3] L. EUZET,[3]
B. MARCHAND,[1] AND T. DE MEEÜS[3,4]

[1] Laboratoire de Parasitologie, Département de Biologie Animale,
Faculté des Sciences de l'Université Cheik Anta Diop, Dakar, Sénégal,
[2] Institute of Parasitic Diseases, Chinese Academy of Preventive Medicine, 207 Rui Jin Er Lu,
Shanghai 200025, People's Republic of China, and
[3] Laboratoire de Parasitologie Comparée, URA 698 CNRS, Université Montpellier II,
Place E. Bataillon, 34095 Montpellier Cedex 05, France

ABSTRACT: The isoenzyme electrophoretic study of 2 species of cestodes, *Avitellina centripunctata* and *Thysaniezia ovilla*, sampled in African (Senegal) domesticated ruminants, revealed a complex of cryptic species. Four species of *Avitellina* were found in sheep and goats and 1 in cattle. Two species of *Thysaniezia*, 1 specific to cattle and the other to sheep, were also revealed. Despite a probable preponderant selfing mode of reproduction, the existence of the detected species was confirmed by high levels of genetic differentiation.

KEY WORDS: Cestoda, *Avitellina, Thysaniezia*, ruminants, isoenzyme electrophoresis, specificity.

Avitellina centripunctata (Rivolta, 1874) Gough, 1911, and *Thysaniezia ovilla* (Rivolta, 1878) Skrjabin, 1926, are 2 cestode species found in the small intestine of numerous herbivorous mammals (Schmidt, 1986). In domesticated ruminants, numerous other species of these two genera of Anoplocephalidae have been described by different authors, none of which is valid (Spasskii, 1951; Troncy et al., 1981). It is difficult to explain this lack of parasite diversity considering the heterogeneity of potential hosts and their diets.

In this article, we present a population genetic study, based on isoenzyme electrophoresis, of African (Senegal) *A. centripunctata* and *T. ovilla*, sampled in sheep, goats, and cattle. This enabled us to test the genetic homogeneity and the degree of specificity within the 2 cestode species. This study revealed a broader diversity of species and a narrower range of host specificity than suspected.

Material and Methods

Sampling of the worms

Two morphological species of parasite were studied, *Avitellina centripunctata* and *Thysaniezia ovilla* (Cyclophyllidea: Anoplocephalidae). Cestodes were taken from the small intestine of cattle ($n = 30$), sheep ($n = 80$), and goats ($n = 38$) from the Dakar (Senegal) slaughterhouse during the summer of 1992. For *A. centripunctata*, prevalences were 15, 8, and 7% in sheep, goats, and cattle, respectively. For *T. ovilla*, prevalences were 6 and 13% in sheep and cattle, respectively (goats not infected). The exact origin of each host is unknown; they may come from any region of the northern part of Senegal. Parasites were kept alive in physiological saline (0.9% w/v NaCl). After being identified under a dissecting stereoscope, the cestodes were stored in liquid nitrogen. It is known that such treatment prevents the contamination with host enzymatic material (e.g., Nadler, 1987; Johnson and Hoberg, 1989; Chilton et al., 1992). Parasites were then carried to Montpellier (France) on dry ice.

Preparation of the worms

In the laboratory, worms were thawed. One portion was fixed in alcoholic Bouin fixative, stained with aceto-carmine, mounted in Canada balsam (Martoja and Martoja, 1967), and observed under a light microscope. This enabled a precise diagnosis of the cestodes, using the criteria described by Schmidt (1986). At this time, no morphological heterogeneity could be found within each of the 2 species. Another portion of each parasite, corresponding to a volume of 0.5 ml, was homogenized in Eppendorf tubes filled with an equal volume of distilled water, centrifuged at 12,000 rpm for 1 min, and the homogenates were used as the protein source.

Electrophoresis

Starch gel electrophoresis was performed as described by Renaud and Gabrion (1988). The enzyme systems studied and their corresponding Enzyme Commission numbers were as follows: glucose phosphate isomerase (GPI, EC 5.3.1.9), hexokinase (HK, EC 2.7.1.1), malate dehydrogenase (MDH, EC 1.1.1.37),

[4] Corresponding author: Thierry De Meeüs, Laboratoire de Parasitologie Comparée, URA 698 CNRS, Université Montpellier II, Place E. Bataillon, 34095 Montpellier Cedex 05, France.

Table 1. Genotypes observed for *Avitellina centripunctata*. The 4 genetic entities observed (A1, A2, A3, and A4) are shown separately. The cestodes came from 3 host species: sheep, goat, and cattle (represented by the third letter S, G, and C, respectively). Alleles were numbered according to their anodal mobility.

	GPI	MDH	PEP-A	HK	NP	PGM	ME	*N**
A1S1	2/2	1/1	2/2	3/3	4/4	2/2	2/2	9
A1S2	2/2	1/1	4/4	3/3	4/4	2/2	2/2	1
A1G1	2/2	1/1	4/4	3/3	4/4	3/3	2/2	1
A2S1	3/3	3/3	5/5	4/4	2/2	1/1	1/1	2
A2G1	3/3	3/3	5/5	4/4	2/2	1/1	1/1	2
A2S2	3/3	3/3	5/5	3/3	2/2	1/1	1/1	1
A2G2	3/3	3/3	4/4	4/4	2/2	1/1	1/1	1
A2G3	3/3	3/3	5/5	4/4	2/2	1/1	3/3	1
A3S1	3/3	2/2	3/3	2/2	3/3	3/3	3/3	7
A3G1	3/3	2/2	3/3	2/2	3/3	3/3	3/3	1
A3S2	3/3	2/2	3/3	1/1	3/3	3/3	3/3	1
A3S3	3/3	2/2	3/3	1/1	3/3	3/3	4/4	1
A4C1	1/1	2/2	1/1	5/5	1/1	4/4	1/1	10

* Number of individuals.

malic enzyme (ME, EC 1.1.1.40), mannose phosphate isomerase (MPI, EC 5.3.1.8), nucleoside phosphorylase (NP, EC 2.4.2.1), phosphoglucomutase (PGM, EC 2.7.5.1), and peptidase A (PEP-A, EC 3.4.1.1). The number of worms analyzed and the host species from which they came are given in Tables 1 and 2.

Results

Species diversity

The different genotypes obtained are presented in Tables 1 and 2 for *A. centripunctata* and *T. ovilla,* respectively. It can be noted that the 2 "species" actually correspond to 2 species complexes. *Avitellina centripunctata* is subdivided into 4 genetically distant species. Out of the 7 loci studied, each species displayed a level of fixed allelic differences ranging from 71 to 100% (Table 1). Two species, discriminated with 7 (out of 8) diagnostic loci (87% of fixed differences), were observed for *T. ovilla* (Table 2).

Parasite specificity

In the *A. centripunctata* complex (Table 1), parasite specificity isolates the small ruminants (sheep and goats) from the large ruminants (cattle), with 1 species (A4) specific to cattle and the remaining 3 found in sheep and goats. For *T. ovilla,* the 2 species observed displayed a strict specificity (Table 2) for cattle and sheep.

Within-species diversity

Within-species genetic heterogeneity could only be found in *A. centripunctata* species infecting small ruminants (Table 1). This heterogeneity is represented by rare alleles in several of the loci studied. These loci are PEP-A and PGM for species A1; PEP-A, HK, and ME for species A2; and HK and ME for species A3 (Table 1). Within outcrossing species, homozygosity is highly unlikely for rare alleles (more likely to be found at a heterozygous stage) (Hartl and Clark, 1989). No heterozygote could be found, even for rare alleles. This strongly suggests that selfing may be the preponderant mode of reproduction for these cestodes.

Discussion

As demonstrated in similar studies, parasite species diversity is often much more complex

Table 2. Genotypes obtained for *Thysaniezia ovilla* from sheep (TS) and cattle (TC). Alleles were numbered according to their anodal mobility.

	MDH	PEP-A	NP	PGM	ME	HK	MPI	GPI	*N*
TS	1/1	2/2	2/2	2/2	1/1	1/1	1/1	1/1	14
TC	2/2	1/1	1/1	1/1	2/2	2/2	2/2	1/1	16

* Number of individuals.

than what morphological taxonomy has previously postulated. This is true for various kinds of parasitic organisms: cestodes (Renaud et al., 1983; Renaud and Gabrion, 1984, 1988; de Chambrier et al., 1992), trematodes (Reversat et al., 1989), nematodes (Nascetti and Bullini, 1982; Andrews et al., 1989; Chilton et al., 1992), acanthocephalans (de Buron et al., 1986), and caligid copepods (Zeddam et al., 1988).

The level of biological diversity characterized within the cestodes studied appeared much higher than what has been reported (e.g., Euzéby, 1966; Soulsby, 1968; Troncy et al., 1981; Schmidt, 1986). Specificity was found to separate worms infecting small ruminants (sheep and goats) from those found in large ruminants (cattle). For certain kinds of organisms, in particular cestodes, selfing may make species characterization more difficult (Lymbery, 1992). Here, the high levels of genetic differentiation strongly validate the 6 species characterized, even for those represented by few individuals.

Species that self are likely to display high heterozygote deficiencies and, thus, low levels of polymorphism (homozygosity lowers the effective population size, i.e., accelerates drift) (Li, 1976). Accordingly, no variation was found within the 2 cryptic species of *Thysaniezia* and within 1 *Avitellina* species (cattle parasite). Some loci studied appeared polymorphic within 3 *Avitellina* species. No heterozygous individuals could be observed within these 3 species. Hosts probably came from a wide area. However, some migration must occur due to human activity (host migrations). Attributing the observed absence of heterozygotes to population structuring would require a total geographical isolation between the different units (no migration). High levels of selfing thus represents a suitable explanation.

In the small intestine of African domesticated ruminants, species of *Avitellina* and *Thysaniesia* coexist with other cestodes: *Stilesia globipunctata, Moniezia expansa,* and *M. benedeni* (e.g., Euzéby, 1966), some of which are themselves species complexes (unpubl. data). The ecological factors allowing such a species diversity remain unknown. However, differences in intermediate hosts and in host grazing behaviors may explain heterogeneities in host infections. The goats, for example, were rarely infected, compared to sheep. Moreover, it is probable that all these coexisting species display different ecological and transmission strategies. This remains to be studied. It is probable, as well, that the effect of anthelmintic treatments is different on these different parasite species. Consequently, the control of these diseases of veterinary importance may be more complicated than expected.

Literature Cited

Andrews, R. H., I. Beveridge, M. Adams, and P. R. Baverstock. 1989. Genetic characterization of three species of *Onchocerca* at 23 enzyme loci. Journal of Helminthology 63:87–92.

de Buron, I., F. Renaud, and L. Euzet. 1986. Speciation and specificity of acanthocephalans. Genetic and morphological studies of *Acanthocephaloides geneticus* sp. nov. parasitizing *Arnoglossus laterna* (Bothidae) from the Mediterranean littoral (Sète-France). Parasitology 92:165–171.

de Chambrier, A., C. Vaucher, and F. Renaud. 1992. Etude des caractères morpho-anatomiques et des flux géniques chez quatre *Proteocephalus* (Cestoda: Proteocephalidae) parasites de *Bothrops jararaca* du Brésil et description de trois espèces nouvelles. Systematic Parasitology 23:141–156.

Chilton, N. B., I. Beveridge, and R. H. Andrews. 1992. Detection by allozyme electrophoresis of cryptic species of *Hypodontus macropi* (Nematoda: Strongyloidea) from macropodid marsupials. International Journal for Parasitology 22:271–279.

Euzéby, J. 1966. Les Maladies Vermineuses des Animaux Domestiques et leurs Incidences sur la Pathologie Humaine. Tome II, Maladies dues aux Plathelminthes. Fasc. 1: Cestodoses. Vigot Frères eds., Paris. 663 pp.

Hartl, D. L., and A. G. Clark. 1989. Principles of Population Genetics, 2nd ed. Sinauer Associates, Sunderland Massachusetts. 682 pp.

Johnson, M. R., and E. P. Hoberg. 1989. Differentiation of *Moniezia expansa* and *Moniezia benedeni* (Eucestoda: Cyclophyllidea) by isoelectric focusing. Canadian Journal of Zoology 67:1471–1475.

Li, C. C. 1976. First Course in Population Genetics. The Boxwood Press, Pacific Grove, California. 631 pp.

Lymbery, A. J. 1992. Interbreeding, monophyly and the genetic yardstick: species concepts in parasites. Parasitology Today 8:208–211.

Martoja, R., and Martoja, M. 1967. Initiation aux Techniques de l'Histologie Animale. Masson, Paris. 329 pp.

Nadler, S. A. 1987. Biochemical and immunological systematics of some ascaridoid nematodes: genetic divergence between congeners. Journal of Parasitology 73:811–816.

Nascetti, G., and L. Bullini. 1982. Biochemical taxonomy of some ascaridoid nematodes. Molecular and Biochemical Parasitology 34:7–14.

Renaud, F., and C. Gabrion. 1984. Polymorphisme enzymatique de populations du groupe *Bothriocephalus scorpii* (Mueller, 1776) (Cestoda, Pseudophyllidea). Etude des parasites de divers Téléostéens des côtes du Finistère. Bulletin de la Société Française de Parasitogie 2:95–98.

———, and ———. 1988. Speciation in cestoda. Evidence for two sibling species in the complex *Both-*

rimonius nylandicus (Schneider 1920) (Cestoda: Cyathocephalidea). Parasitology 97:1–9.

———, ———, **and N. Pasteur.** 1983. Le complexe *Bothriocephalus scorpii* (Mueller, 1776): differentiation par électrophorèse enzymatique des espèces parasites du Turbot (*Psetta maxima*) et de la Barbue (*Scophthalmus rhombus*). Comptes Rendus de l'Académie des Sciences de Paris 296: 127–129.

Reversat, J., F. Renaud, and C. Maillard. 1989. Biology of parasites populations: the differential specificity of the genus *Helicometra* Odhner, 1902 (Trematoda: Opecoelidae) in the Mediterranean Sea demonstrated by enzyme electrophoresis. International Journal for Parasitology 19:885–890.

Schmidt, G. D. 1986. Handbook of Tapeworm Identification. CRC Press, Boca Raton, Florida. 675 pp.

Soulsby, E. J. L. 1968. Helminths, Arthropods and Protozoa of Domesticated Animals. Baillère, Tindall and Cassel Ltd Eds., London. 824 pp.

Spasskii, A. A. 1951. Essentials of Cestodology. Vol 1: Anoplocephalate Tapeworms of Domestic and Wild Animals. Academy of Sciences of the USSR, Moscow. 783 pp.

Troncy, P. P., J. Itard, and P. C. Morel. 1981. Précis de Parasitologie Vétérinaire et Tropicale. Editions du point vétérinaire, Maisons-Alfort. 328 pp.

Zeddam, J. L., P. Berrebi, F. Renaud, A. Raibaut, and C. Gabrion. 1988. Characterisation of two species of *Lepeophtheirus* (Copepoda, Caligidae) from flatfishes. Description of *Lepeophtheirus europaensis* sp. nov. Parasitology 96:129–144.

Meeting Schedule

HELMINTHOLOGICAL SOCIETY OF WASHINGTON 1994

(Wednesday) 9 February 1994	Animal Parasitology Unit, U.S. Department of Agriculture, Beltsville, MD
(Wednesday) 6 April 1994	Johns Hopkins University, Baltimore, MD
(Saturday) 7 May 1994	Joint Meeting with the New Jersey Society for Parasitology, at the New Bolton Center, University of Pennsylvania, Kennett Square, PA
October 1994	Site to be announced
November 1994	Site to be announced